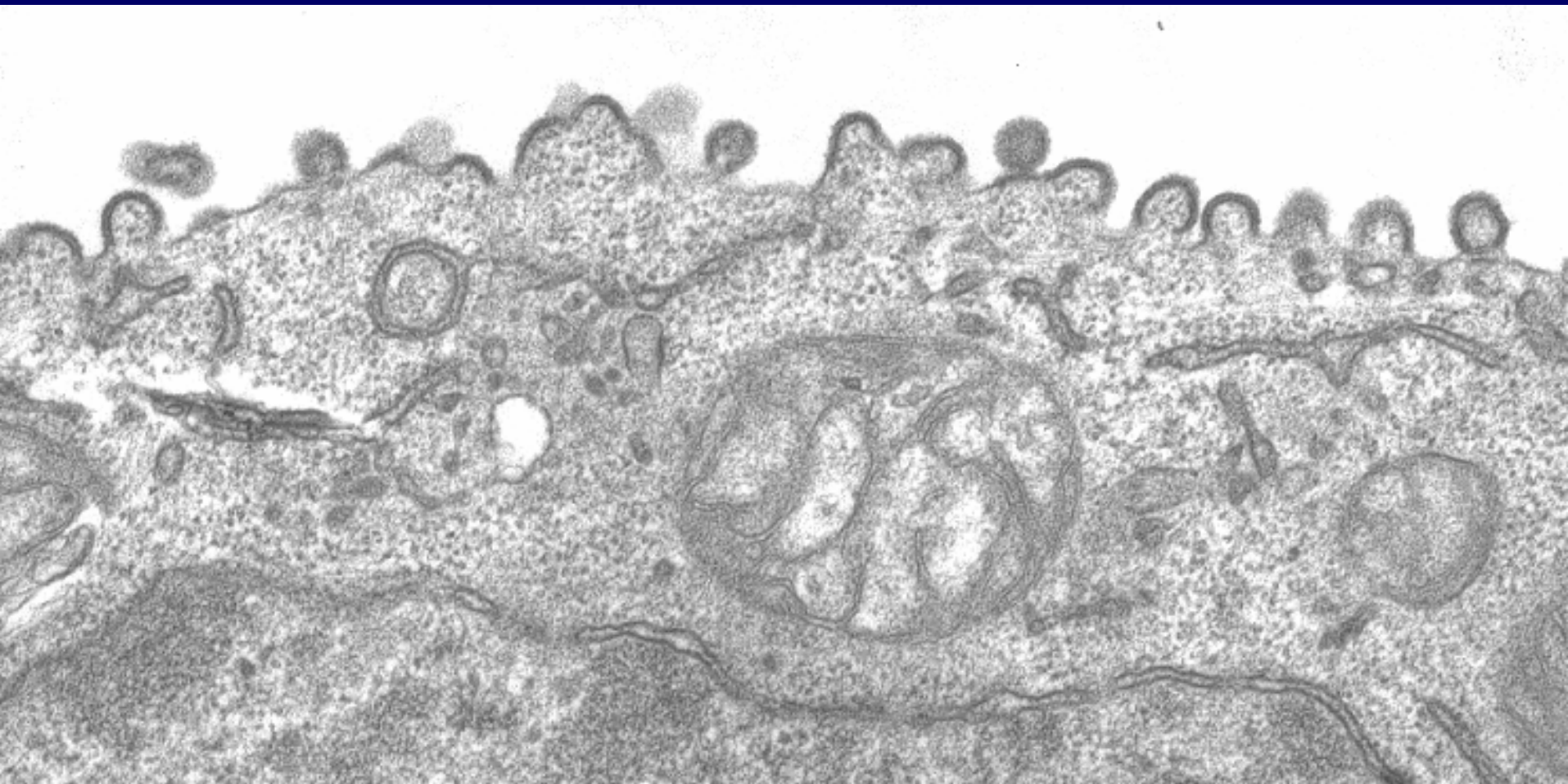
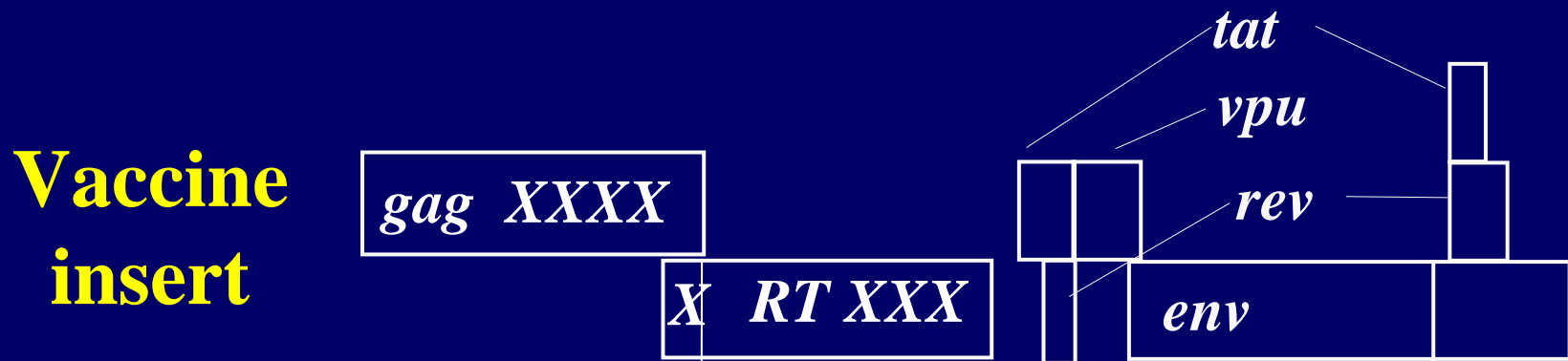


In vitro and *in vivo* dose
response studies for DNA,
MVA, and DNA/MVA
vaccines

**Both DNA and MVA express
non-infectious HIV-like particles**



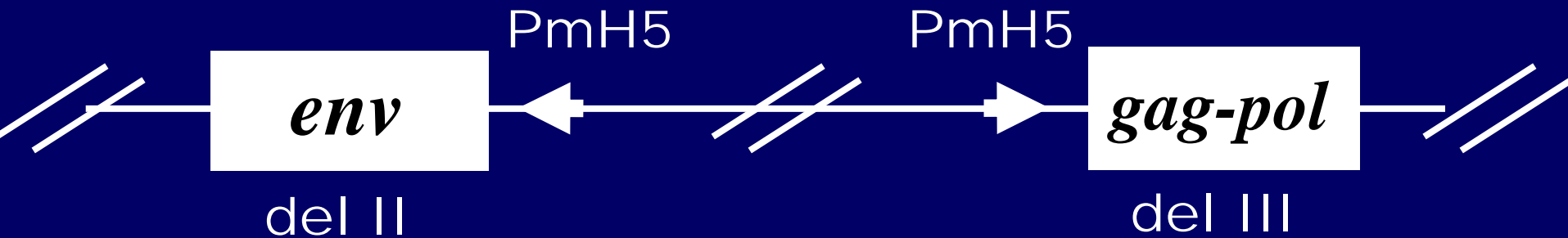
Single DNA expresses 6 proteins by subgenomic splicing



Deletions and safety mutations render non-infectious

Developed by Emory Vaccine Center and CDC

Single MVA expresses Gag- Pol and Env

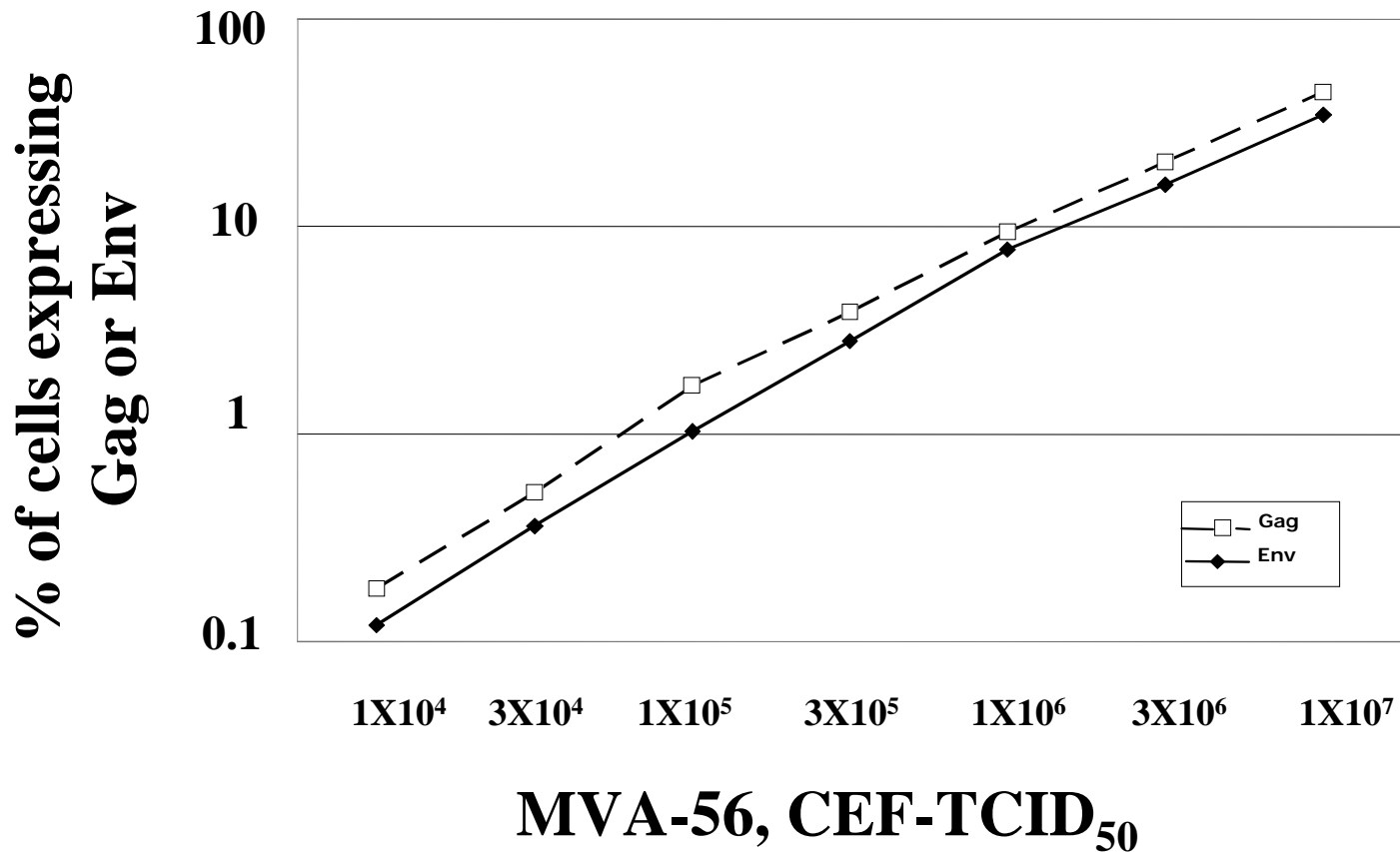


Developed by Bernie Moss, Linda Wyatt, pate Earl
At NIAID

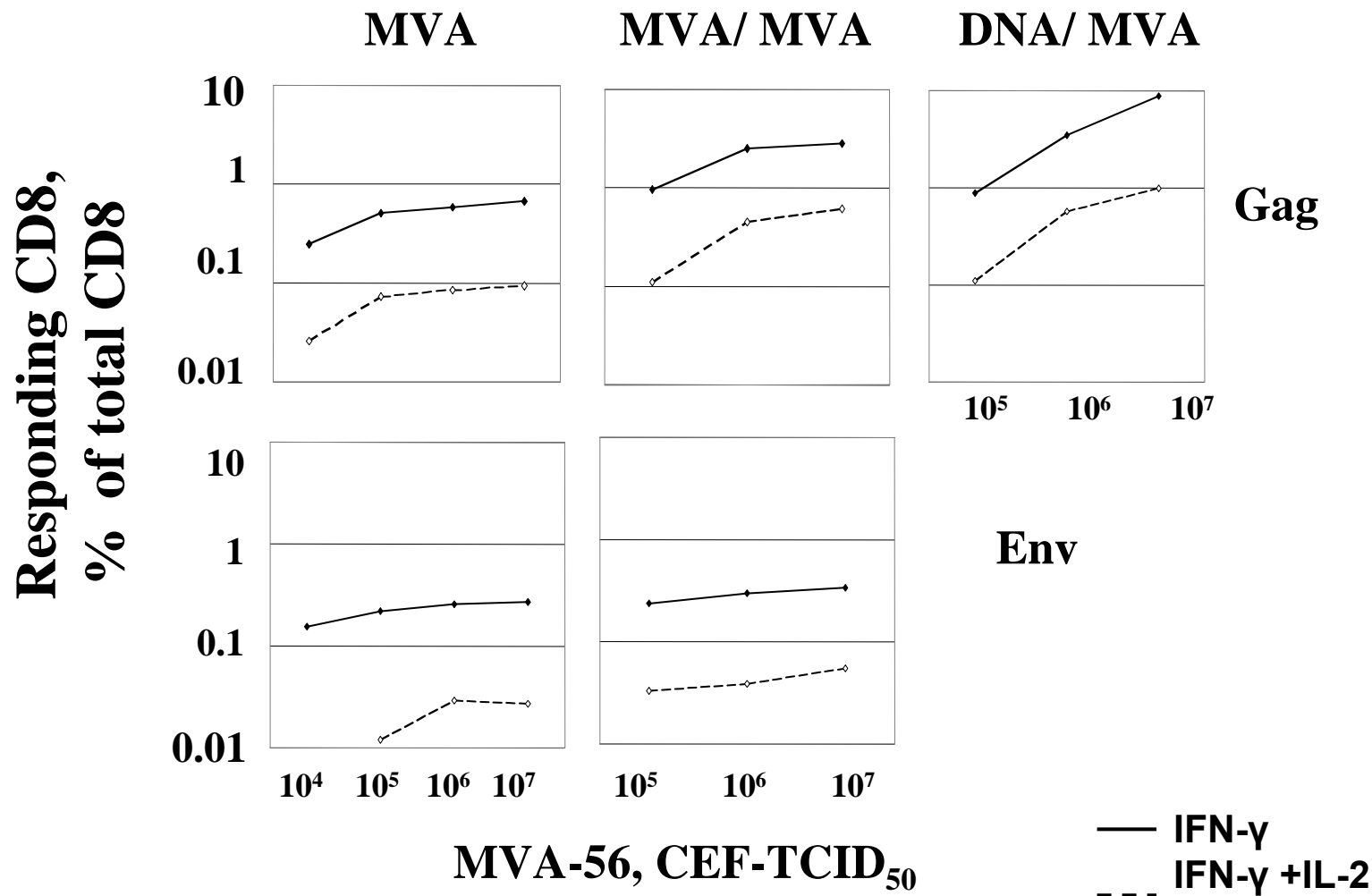
Tests done on MVA

- *In vitro* expression test – infection of 293T cells and FACS analyses for Gag or Env expressing cells
- *In vivo* immunogenicity test – i.m. inoculations into Balb/c mice, intracellular cytokine analyses for CD8 and CD4 responses used pooled peptides for stimulating pooled splenocytes

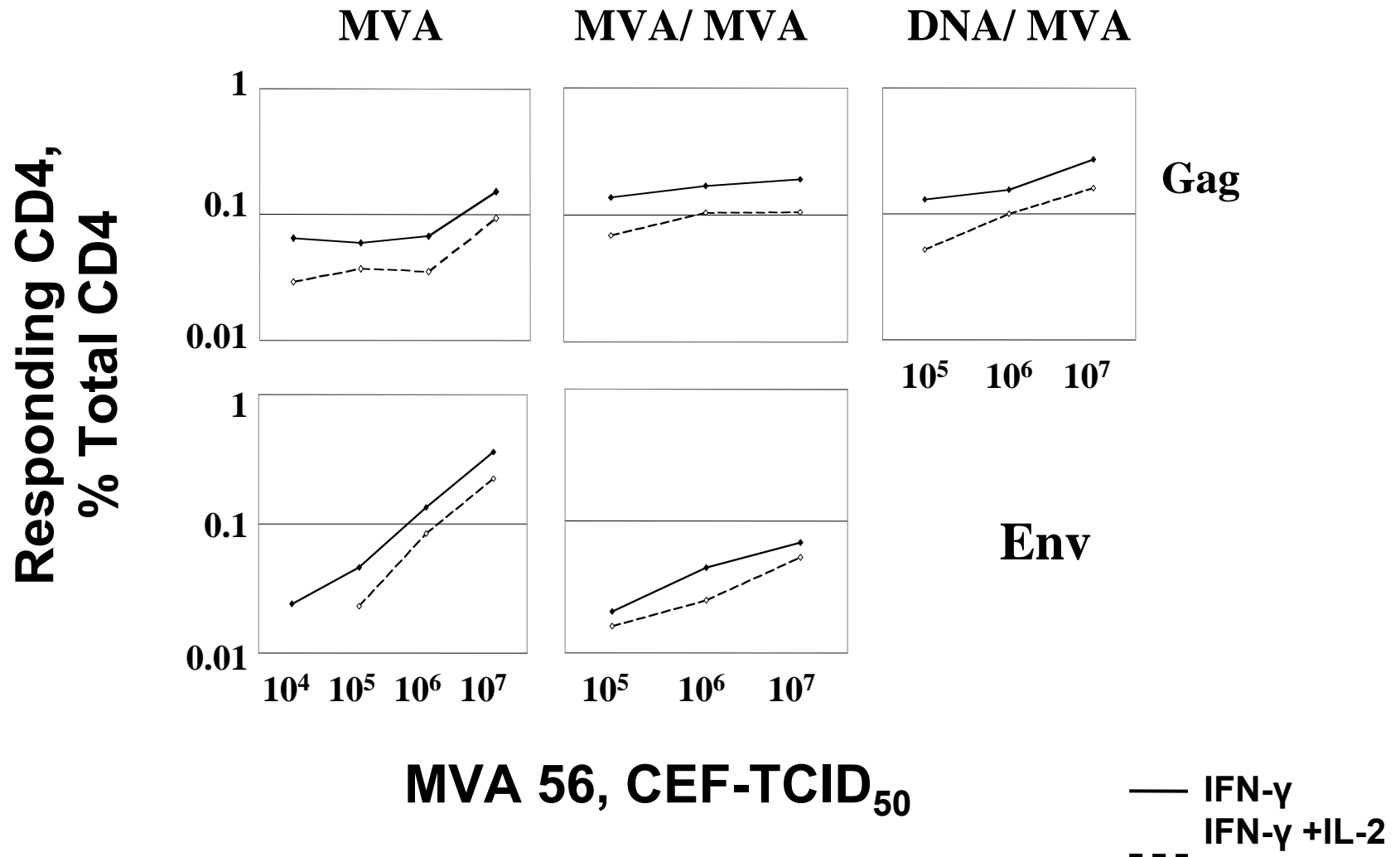
In vitro MVA expression



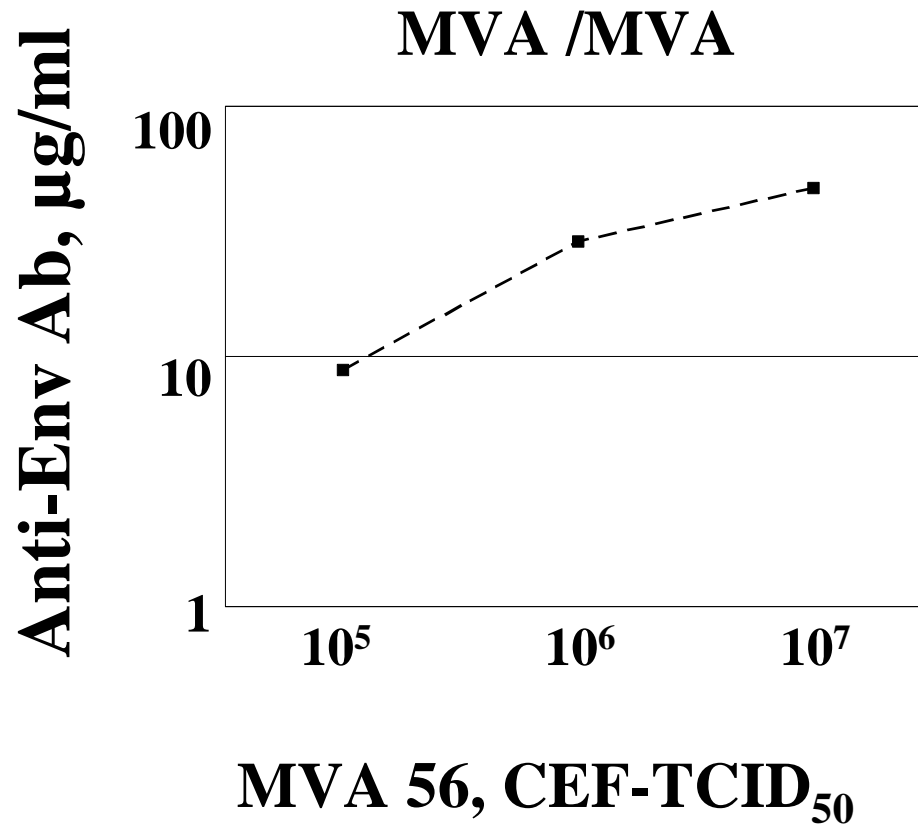
In vivo MVA immunogenicity - CD8 cells



In vivo MVA immunogenicity, CD4 cells



In vivo MVA immunogenicity - Ab



Summary for MVA

- *In vitro* expression – much sharper dose response for biological activity than *in vivo* immunogenicity
 - *In vitro*: 1000-fold increase in MVA dose – 300-fold increase in expressing cells
 - *In vivo*, post prime, boost, or as a boost for a DNA prime – ≤ 10 fold increases in immunogenicity for ≥ 100 -fold increases in doses

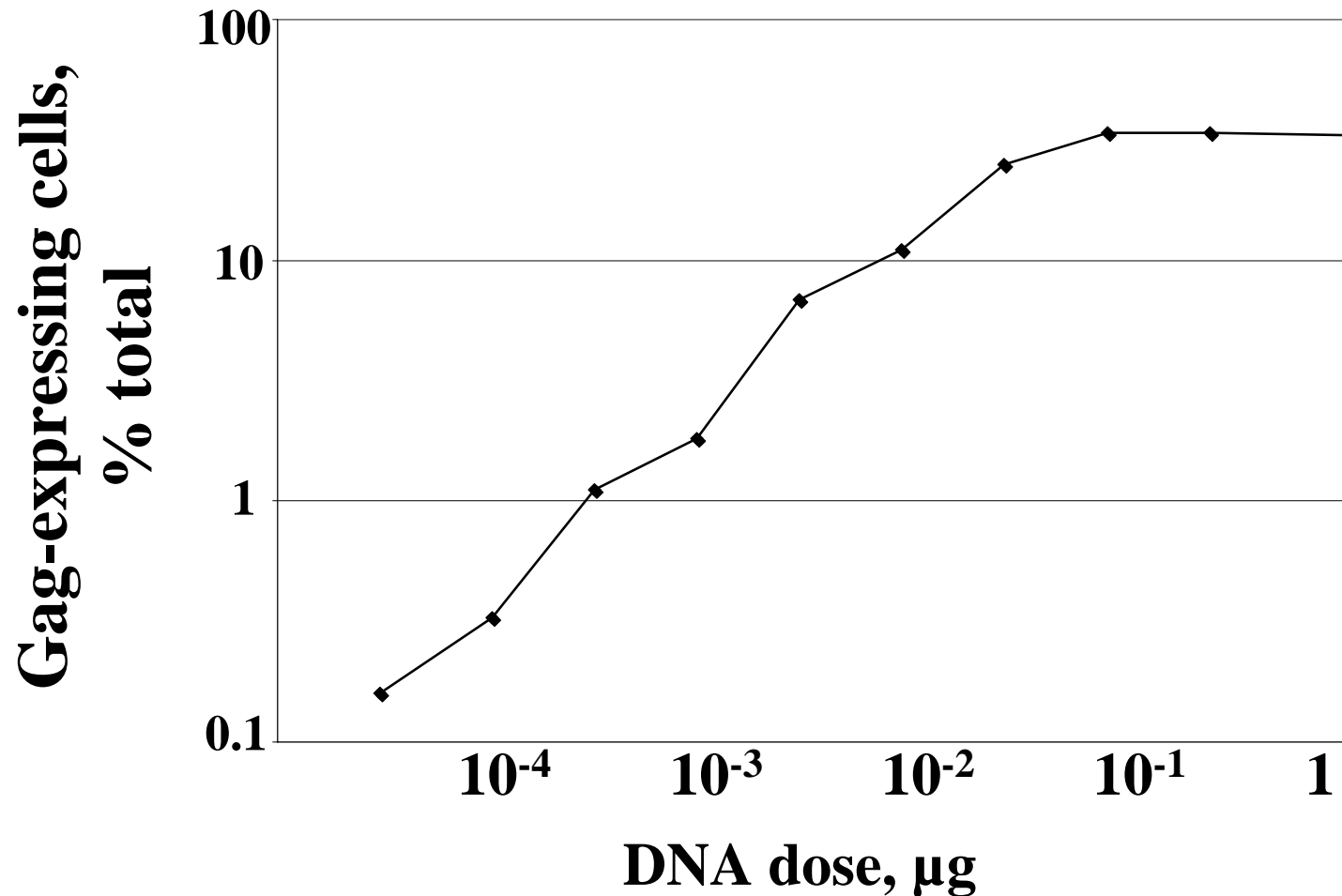
Conclusions from MVA

- Use *in vitro* test for lot to lot comparisons, stability analyses
- *In vivo* tests (prime, prime and homologous boost, heterologous prime/boost) show that dose affects response, but not suited to accurately following lot to lot variability

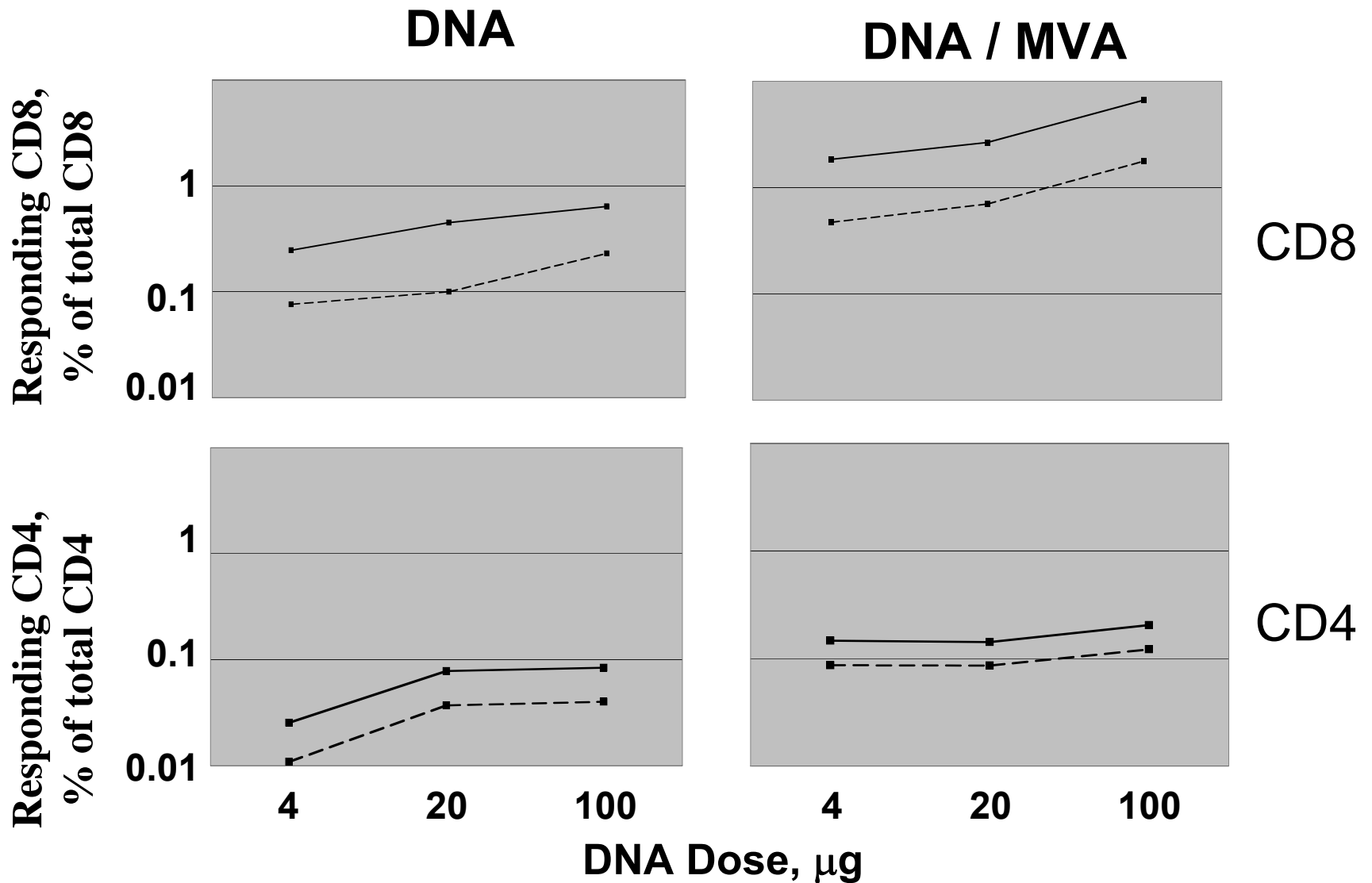
Tests done on DNA

- Used codon-optimized Gag expressing DNA (VLP-expressing DNA does not express well in mouse cells)
- DNA, >80% covalently closed circular
 - *In vitro*-test – transient transfection and FACS analyses for Gag expressing cells
 - *In vivo* test – i.m. inoculations into Balb/c mice, intracellular cytokine analyses for CD8 and CD4 responses used pooled peptides to stimulate pooled splenocytes

In vitro DNA expression



In vivo DNA immunogenicity, post prime and boost



Summary for DNA

- *In vitro* expression – much sharper dose response for biological activity than *in vivo* immunogenicity
 - *In vitro*: 80-fold increase in DNA – 33-fold increase in expressing cells
 - *In vivo*, post prime or heterologous boost: 25-fold increase in DNA – 2-4 fold increase in responding CD8 or CD4 T cells

Conclusions from DNA

- Use *in vitro* test for lot to lot comparisons, stability analyses
- *In vivo* tests (prime and heterologous prime/boost) show that dose affects response, but not suited to accurately following lot to lot variability

In vivo versus *in vitro* tests

- Both DNA and MVA
 - More accurate lot to lot assessments and stability assessments of biological activity using *in vitro* expression than *in vivo* immunogenicity
 - FACS analyses – highly quantitative *in vitro* assessments of vector stability and vector expression of more than one vaccine antigen

Acknowledgements

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